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Molecular docking study to elucidate the anti-pruritic mechanism of selected natural ligands by desensitizing TRPV3 ion channel in Psoriasis: An *in silico* approach

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Psoriasis is a chronic immune-mediated inflammatory skin disease, in which pruritus is a common feature and also affects the social well-being of individuals with psoriasis significantly. The transient receptor potential cation channel, subfamily V, member 3 (TRPV3) is believed to be involved in hypersensation and generation of itching in the case of psoriasis. The purpose of the present study was to find out suitable anti-pruritic agents and to establish the mechanism of actions of those anti-pruritic agents with the help of molecular docking studies, through which they can alleviate the itching and hypersensitivity problems in psoriasis. An extensive literature survey, pertaining to natural ligands having reported antipsoriatic activity was carried out. The crystal structure of the TRPV3 receptor was retrieved from rcsb.org. 3D structures of selected eleven natural ligands were prepared and optimized by ChemSketch free version 2015. Computational protein-ligand docking studies were carried out by AutoDock 4.2 simulator using the Lamarckian genetic algorithm. In this study, Hypericin showed a higher binding affinity (–8.09 kcal/mol) and fitted into the active pocket of TRPV3. Results revealed that Hypericin might be the candidates to be employed as an anti-pruritic agent in the case of psoriasis to desensitize the TRPV3 ion channel.

Keywords: Antipruritic activity, Binding affinity, Natural Ligands, Psoriasis, TRPV3

Psoriasis is a common and chronic autoimmune skin disease[1], in which not only skin but other organ systems *viz*. gastro-intestine tract, musculoskeletal system, and eye are also inflamed[2]. Itch (pruritus) is an important symptom of psoriasis which significantly impacts the social life of individuals with psoriasis. Approximately 70-80% of individuals with psoriasis have an itch problem[3]. TRPV3 channels, a type of TRP (transient receptor potential cation channel subfamily V member 3), are expressed predominantly on keratinocytes, play a pivotal role in the generation of itch[4-5]. TRPV3 is a thermo-sensitive channel to be activated by changes in the environmental temperature and also activated by chemicals[6]. The reason of itch generation after TRPV3 activation is due to the release of mediators from keratinocytes (IL-1 α, ATP or PGE2) or which induce TRPV3 sensitization (Bradykinin, PGE2, Histamine, and ATP). As these factors, via the TRPV3-coupled (and possibly other) signaling mechanisms, may further augment the release of one another from skin cells, their functions additionally support the role of TRPV3 in itch[7]. Apart from that dysfunction of TRPV3 channels leads to various skin diseases and over expression of these channels is responsible for the development of lung cancer and colorectal disease. In recent times TRPV3 has been identified as a better target for drug development for pain, itch, and different skin ailments, but the limitations of apprehensiveness with respect to the role of this novel target in pathophysiology of diseased conditions and lack of structural information of TRPV3 also affected the drug discovery process[8].To improve the social well-being of patients suffering from psoriasis many agonists and antagonists have been identified. Therefore, this study aims at finding some suitable TRPV3 antagonists so that pruritus and other cardinal symptoms of psoriasis might be treated. In the present study, eleven herbal molecules (*i.e.*Caffeine[9], Catechin[10], Curcumin[11], Embelin[12], Epicatechin[13], Gossypol[13,14], Hypericin[15], Luteolin[16], Quercetin[17,

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Dimethyl Fumerate and Monoethyl Fumerate[18]), which have shown antipsoriatic activity preclinically, were included and their interactions with molecular target were demonstrated by molecular docking study to elucidate their molecular mechanism of action to inhibit the pruritus in psoriasis. This study will be helpful to support the role of TRPV3 in the pathophysiology of pruritus in psoriasis and other skin diseases.

Methods and Materials

Retrieval of Molecular Target

The crystal structure of the molecular target (TRPV3, PDB id: 6dvz) which is engaged in the generation of pain and itch in psoriasis was retrieved from RCSB protein data bank[8].

Target optimization

Macromolecule needs to be prepared, prior to docking process. Preparation involves the removal of water molecules and any unwanted hetero atoms, because these may interfere with the docking process. After refining, macromolecule was saved as a .pdb execution file. The macromolecule was loaded and stored as macromolecules.pdbqt after assigning hydrogen bonds and Gasteiger charges.

Ligand preparation and optimization

Investigational ligands were designed using ChemSketch (ACD 2015) and optimized for energy minimization using the MM2 force field (Fig. 1). The ligands were loaded and their torsions along with rotatable bonds are assigned and the files are saved as ligand.pdbqt.

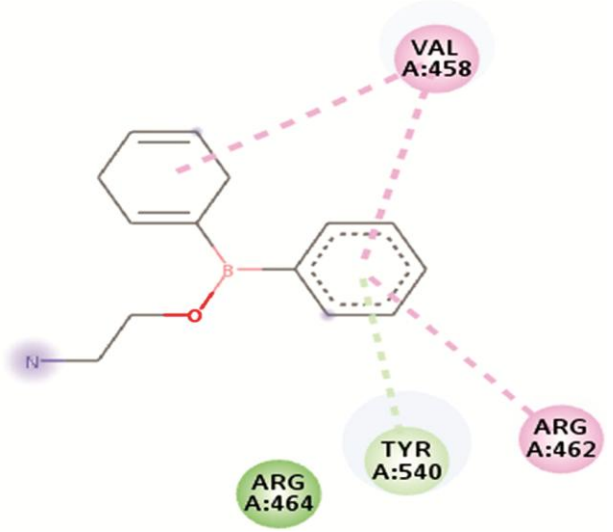

Fig. 1 — 2-APB at Site4 on which it might be behaving like the antagonist

Molecular docking analysis

To confirm the binding modes of compounds undertaken in our research with receptor protein, molecular docking studies were carried out using AutoDock v 4.2.6 software. In this way, the different conformers were generated of the compounds and blind docking was performed on molecular target and the best conformers were discussed with the lowest binding energy (–kcal/mol). The blind docking was performed to find out the actual binding site of antipsoriatic molecules which might pave the way to disclose the mode of actions of natural ligands. Conformers of the ligands were automatically docked to the target, Transient receptor potential cation channel. The docking parameters were defined as coordinates of the center of binding site with x = 126, y = 126, z = 126,and binding radius = 0.375 Å and Coordinates of Central Grid Point of Maps were X=80.411, Y=120.810, and Z=115.292. All AutoDock output file (.dlg) were then analyzed through Analysis option provided in MGLTools-1.5.6 rc3. Top-scoring molecules in the largest cluster were analyzed. Complexes (in .pdbqt format) of the different docked conformers of the ligands with the protein were manually prepared and converted to .pdb format through pdbqt_to_pdb.py script. Rigid dockings were performed using Genetic Algorithms and keeping other docking parameters in default. Followed by, setting up of docking parameter files with the search parameter as a genetic algorithm and docking parameter utilizing a Lamarckian genetic algorithm. The Lamarckian genetic algorithm (LGA) was applied to deal with all the proteins–ligands interactions. The docked structures of the inhibitors were generated after a maximum number of evaluations.

Results

The crystal structure of TRPV3 was composed of four subunits therefore in this study only a single unit was used for molecular docking purposes. Recently Singh *et al*. 2018 elaborated on the gating mechanism of TRPV3 channels and hypothesized that responsible site for target desensitization is site 4, composed of V458, Q483, L484, R487, M488, V490, L491, V537, Y540, and L551 and critical for channel opening[8]. Therefore in the current study, site 4 in the target channel was chosen for the molecular docking study of the natural ligands. Most of the ligands included in the study, except Dimethyl Fumerate and Monoethyl Fumerate, perfectly interacted to residues of the active site (Site 4) of the macromolecule with

different affinities and produces some conformational changes in the TRPV3 channel and subsequently modulated channel functioning and desensitization of predominantly expressed TRPV3 channels responsible for the itchy skin. Figure1 shows that 2-APB interacted with Valine 458, Arginine 462, Arginine 464, Tyrosine 540, On Transient receptor potential cation channel, subfamily V, member 3 (TRPV3) and indicated antagonistic activity. Therefore, it is essential that investigational ligands must also have some interactions with those amino acid residues. Hypericin showed the highest binding affinity *i.e*. –8.09 kcal/mol in comparison to other ligands and formed three hydrogen bonds with target residues *i.e*. Oxygen molecule of Tyrosine 540 (1.909Å and 1.922 Å) and NH molecule of Arg 464 (1.771Å) residues of A chain. In this molecular docking study, Catechin was the only flavonoid which exhibited moderate binding energy (−6.57 kcal/mol) and afforded the formation of three hydrogen bonds with Oxygen molecule of Tyrosine 540 (1.91 Å), Oxygen molecule of Proline 470 (1.91 Å) and NH of Arginine 464 (2.015 Å) of A chain of target channel whereas Luteolin, Epicatechin and Quercetin demonstrated low binding affinities (−5.42, −5.32 and 5.69 kcal/mol, respectively) and could not afford the prerequisite hydrogen bonds with TRPV3 ion channel. Luteolin formed h- bond with Histidine 471 residue (2.056 Å), Epicatechin could form the hydrogen bonds with Leucine 475 (1.907 Å) and Methionine 488 (2.165Å) and Quercetin could not form any hydrogen bond with TRPV3. Other ligands like Gossypol, Curcumin, Embelin and Fumaric esters (Dimethyl and MonoethylFumerate) also exhibited good binding affinity towards the TRPV3 channel (−6.37, −5.34, −4.82, −3.94 and −3.54 kcal/mol, respectively). But, only Curcumin interacted with Arginine 464 (1.937 Å) and Embelin could afford the essential hydrogen bonds Arginine 464 (2.135 Å), Tyrosine 540 (1.877 Å) with target and Fumaric esters could interact with TRPV3 ion channel with different hydrogen bonds like MonoethylFumerate with Lysine 705 (1.799 Å) and Lysine 500 (1.649 Å) and Dimethyl Fumerate with Lysine 705 (1.958 Å) and Lysine 500 (2.06 Å) fitted into the different region of the channel. Overall, Hypericin exhibited better binding affinity to the target channel and bound to the site 4 of 2-APB which may behave like antagonists at this site when undergone molecular docking analysis (Fig. 2).

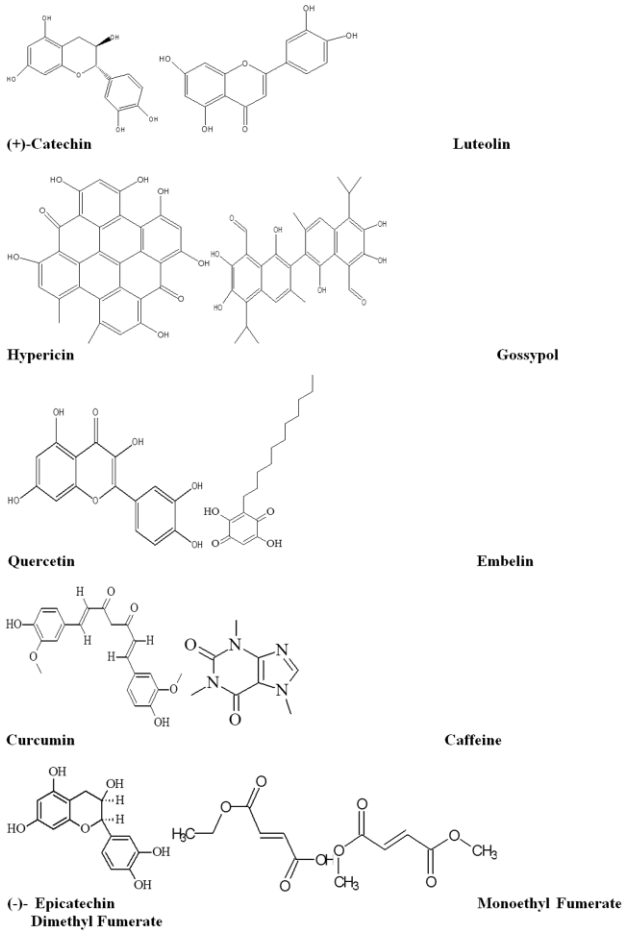

Fig. 2 — Structure of Ligands

Discussion

The present study aimed at evaluation of binding of selected phytoconstituents to TRPV3, in order to find out their possible use in the treatment of pruritis associated with psoriasis. TRPV3, a member of thermosensitive TRP channels, is found to be expressed in keratinocytes. Its involvement in causing itch is well documented. It is activated by warm temperature and repetitive stimulation of agonists like 2-aminoethoxydiphenyl borate (2-APB), Eugenol, Carvacol, and many terpenes. It is identified as an important channel in pruritis and skin disorders such as psoriasis.TRPV3 sensitization takes place due to a reduction in extracellular Ca^{+2} and their subsequent interactions with ASP641 residue at the pore loop and might be responsible for TRPV3 inhibition by some molecules[20]. Luo *et al*. (2012) reported that two acidic residues E679 and E682 located in the inner pore region or a residue ASP641 from the extracellular pore loop that are critical for TRPV3 mediated signaling. These

residues interacted with Mg^{+2} and this ion is responsible for desensitization of TRPV3[21]. Doerner *et al.* (2011) postulated that voltage and temperature- dependent activation of TRPV3 channels is potentiated by receptor-mediated PIP_2 hydrolysis and proposed two residues ARG696 and LYS705 being responsible for inhibition of TRPV3 channels[22]. Moreover, reduced Ca^{+2} dependent inactivation of TRPV3 causing desensitized TRPV3. Xiao (2008) also reported that increased Ca^{+2} ions intracellularly lead to inhibition of TRPV3 channels[23]. However, Singh *et al*. 2018 discovered that the binding of 2-ABP (agonist to TRPV3) at site4 responsible for opening and subsequent desensitization of the channels[8]. Interaction of Hydroxyl groups in the ligands as well as more hydrophobicity and electron donating tendency of these ligands with this channel are accountable for better binding energies. Vali *et al.* (2005) reported the efficacy of topical application of caffeine in the treatment of psoriasis vulgaris affected patients. In this randomized, double-blind, placebo-controlled study 39 patients with plaque psoriasis were included for eight weeks. Topical application of 10% caffeine or placebo three times a day on the either side of the body was done. Results obtained through this study indicated the reduction in the PASI score, measured at the four visits for caffeine treated group in alternate weeks in 8 weeks which were 2.64±2.89, 4.47±3.62, 5.73±4.16, 6.58±4.40 and for placebo-treated group these were 1.45±2.32, 3.04±2.68, 4.02±3.36, 4.43±3, respectively. The overall study concluded that caffeine is an effective, safe, and cheap option for the treatment of psoriasis[9]. Kurd *et al*. (2008) determined the efficacy of orally administered curcumin in the prospective clinical trial in case of psoriasis. They found that intention-to-treat analysis response rate was 16.7% and PASI score was found to be 75[11]. Kalyan Kumar *et al.* (2011) reported the effect of Embelin on the production of tumor necrosis factor-α through lipopolysaccharide-induced tumor necrosis factor-α production in mice model and *in vitro* human keratinocyte and also examined the effect of Embelin on production of pro-inflammatory cytokines through acute and chronic skin inflammation using 12-O-tetradecanoyl-phorbol-13- acetate (TPA) induced mouse ear edema. Embelin inhibited the TNF-α production induced by Lipopolysaccharide in dose dependent manner at highest doses (50 mg/kg) exhibited maximum inhibition (73.38%) obtained in this study indicated that Embelin had an inhibitory effect on the production of cytokines and also had anti-inflammatory activity[12]. Shrivastava *et al.* (2009) investigated the efficacy of the plant *Thespesia populnea* (Malvaceae) in the treatment of psoriasis although traditionally this plant has been useful in the case of scabies, ringworm, guinea worm, eczema. In this study, three compounds were isolated from the bark extract of this medicinal plant, which are TpF-1, TpF-2, and TpS-2. Different extracts have also been prepared from this plant‘s bark. Further, these isolated molecules and plant extracts were employed for anti-psoriatic screening using Perry’s scientific mouse tail model. The maximum antipsoriatic activity was expressed by successive pet-ether extract by increasing the orthokerototic region by 25% whereas the TpF-2 exhibited 38% increase in the orthokerotic region[13]. Annamalai *et al*. (2013) confirmed in their study that bark of plant *Thespesia populnea* (Malvaceae) contains (+)-gossypol[14]. Kamuhabwa *et al.* (1999) suggested that Hypericin might be an antipsoriatic candidate as they checked some formulations to overcome the problem of photosensitization of Hypericin. In this study, Hypericin was added either into emulsifier ointment supplemented with Solketal® or in polyethylene glycol and applied on skin of hairless mice for 4 h. After that mice were subjected to irradiation with 500 watt halogen lamp. Results showed that there was no measurable photosensitization that occurred by Hypericin when it was added into PEG ointment or 10 mg/kg Hypericin was administered through Intraperitoneal injection[15]. Weng *et al*. (2014) investigated that pretreatment with Luteolin (10-100 μM) significantly inhibited TNF induced mRNA expression in HaCaT cells responsible for release of three mediators involved in the inflammatory process like IL-6, IL-8 and VEGF in concentration- dependent manner and complete inhibition was achieved at 100 μM conc. of Luteolin[16]. Vijayalakshmi *et al*. 2012 reported that Quercetin at the doses of 50 mg/kg and 25 mg/kg increased the orthokerotosis regions (39.8±1.6 and 32.18±3.10, respectively) in comparison to normal (17.07±3.2) and produced antiproliferative activity (IC_{50} 62.42±10.20 μg/mL) and showed anti-inflammatory effect at 50mg/kg dose due to significant (P <0.001) inhibition in leukocyte migration[17]. Harries *et al*. (2005) reported in their retrospective study, the efficacy of fumaric acid esters (FAEs) which were used in the treatment of severe psoriasis in Northern Europe for over 20 years. In this study, they identified patients who received fumaric acid esters for psoriasis treatment at

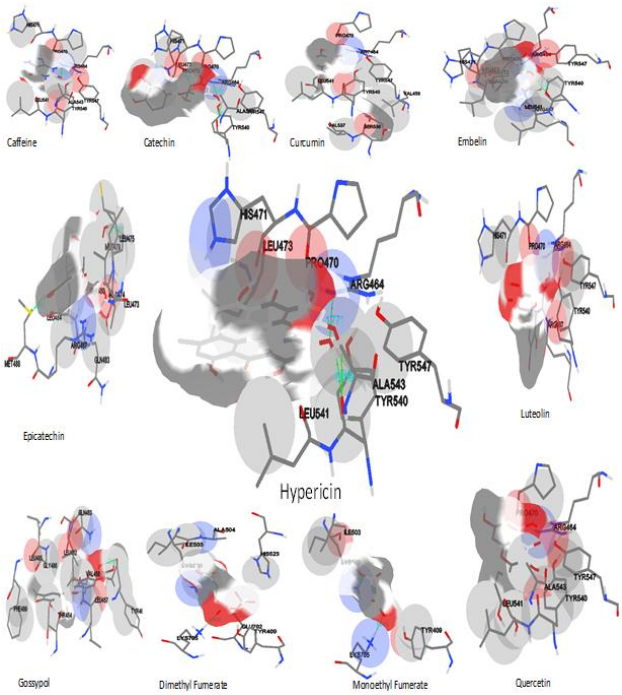

Fig. 3 — Binding modes of Ligands with target TRPV3 (3D model of interactions between ligands and target)

one UK regional referral central between the duration of June 1999 to October 2003. 58 patients were identified (25 women and 33 men), 95% of them had used other systemic antipsoriatic therapy prior to this fumaric acid esters treatment. 32 patients (55%) showed improvement after taking fumaric acid esters with 10 patients (17%) rated clear or virtually clear by physicians. Whereas no improvement was observed in 28% of patients while 16% of patients reported having worsening symptoms of psoriasis after using fumaric acid esters[17]. Waranuch *et al.* (2013) discussed the role of green tea which contains Catechins, Epicatechin, and other ingredients[19]. These contents provide health benefits by free radical scavenging activity and decreasing the extra-cellular matrix degradation induced by ultraviolet rays and other environmental factors like pollution *etc*[18]. Keeping in views all the above studies we have to include phytochemicals to elucidate the mechanism of action of most effective molecule in terms of binding affinity and different interactions with the macromolecule TRPV3. Hypericin has more aromatic rings compared to other ligands; therefore higher hydrophobicity might be a reason to give better binding affinity among all participated natural ligands. Apart from those interactions Epicatechin, Gossypol, and

Table 1 — Amino acids involved in the interactions between Ligands and Target

S. No	Ligand	Amino acids involved in the interactions
1	Caffeine	Arg464, Pro470, His471, Tyr540, Leu541, Ala543, Tyr547
2	Catechin	Arg464, Pro470, His471, Pro472, Leu473, Tyr540, Leu541, Ala543, Tyr547
3	Curcumin	Valine 458, Arg464, Pro470, Serine536, Tyr540, Valine531, Leu541, Tyr547
4	Dimethyl Fumerate	Tyr409, Ile503, Ala504, His523, Glu702, Lys500, Lys705
5	Embelin	Arg464, Pro470, His471, Pro472, Leu473, Tyr540, Leu541,Arg487, Tyr547
6	Epicatechin	Leu473, Ala474, Leu475, Met479, Ser480, Gln483, Leu484, Arg487, Met488
7	Gossypol	Thr 454, Leu457, Val458, Tyr461, Leu482, Gln483, Leu485, Gly486, Phe489
8	Hypericin	Arg464, Pro470, His471, Leu473, Tyr540, Leu541, Ala543, Tyr547
9	Luteolin	Arg464, Pro470, His471, Tyr540, Tyr547, Arg487
10	MonoethylFumerate	Tyr409, Ile503, Lys500, Lys705
11	Quercetin	Arg464, Pro470, Tyr540, Leu541, Ala543, Tyr547

Hypericin could intriguingly make some conformational changes in the ion channel depicted in (Fig. 3 & Table1), whereas, Fumaric esters made quite common changes in the structure of the pore region of the ion channel. All the Flavonoids except Epicatechin, caffeine, Embelin, and Curcumin have made similar sorts of conformational changes in the TRPV3 ion channel. TRPV3 has been involved in the initiation of itch and hyper-sensations in psoriasis therefore in present study overall Hypericin exhibited better binding affinity to the target channel and bound at site 4 in the target and also capable to carry out conformational changes in the target ion channel required for the desensitization of the same when undergone molecular docking analysis. Therefore, this study confirms that Hypericin not only in terms of binding affinity but also due to criterion interactions with amino acids and more essentially capability of carrying out some conformational changes in the ion channel follows the mechanism of action of antagonizing these TRPV3 channels in case of generation of pruritus in person with psoriasis.

Fumaric esters may also act like TRPV3 antagonist because specifically interacted with Lysine 705 as postulated by Doerner *et al*. (2011)

Conclusion

Psoriasis is considered as an untreatable disease due to the lack of understanding of actual pathophysiology. Increasing stress and alteration in environmental conditions are the leading cause of Psoriasis. Psoriasis is attracting the attention of researchers and scientists worldwide due to the increasing numbers of patients. The investigated plant- derived materials have proved efficacy in preclinical testing in the case of psoriasis. Hypericin showed good efficacy in terms of binding affinity and fitted into the binding site and may behave like an antagonist by the desensitizing TRPV3 channel, which is one of the major factors in inducing itching in psoriasis. The plant molecules reported in this communication can be used as prototypes. Many more synthetic molecules can be synthesized for the treatment of itching problems in psoriasis and other skin disorders.

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Conflict of interest

All authors declare no conflict of interest.

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